Detection of Desmin in Formalin-Fixed, Paraffin Embedded Rat Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
DAB Chromagen
Hematoxylin

Antibody Information:

Kit : Vector Rabbit Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: PK-6101
*The Vector Rabbit Elite Kit contains solutions needed to make the secondary and label antibodies.

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-001

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary Antibody: Rabbit anti-desmin
Accurate Chemical & Scientific Corp.
Westbury, NY 11590
(516) 333-2221
www.accuratechem.com
Catalog #YMPS31

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #011-000-001

Staining Procedure

Positive Control Tissue: Smooth and striated muscle cells of the heart
Stain localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Techniques using the microwave oven.
   Place a full rack of slides in tissue tekô container containing 200ml of distilled water.
   Microwave for 5 minutes at level 5
   Cool for 1 minute (Add more distilled water if necessary)
   Microwave for 5 minutes at level 5. Temp after Microwaving_____
   Cool 20 minutes at room temperature.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block with 5% Normal Goat Serum for 20 minutes at room temperature.
   Lot #_____ Reconstituted Date_____

6. Apply Avidin/Biotin block
   Lot#_____ Exp. Date _____New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X AB.
   Apply biotin block - 15 minutes at room temperature.
   Wipe excess block.

7. Apply primary antibody (Desmin) at 1:500 dilution and incubate for one hour at room temperature.
   Lot # _____ Exp date_____

   For negative control slides, normalize the protein concentration of the normal rabbit serum with the protein concentration of the primary antibody (Desmin) and use this to make the 1:500 dilution. Apply to slides and incubate for one hour at room temperature.
   Lot#_____ Reconstituted Date_____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody and incubate for 30 minutes at room temperature.
   Exp date_____New Kit: yes / no

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label antibody and incubate for 30 minutes at room temperature.
    (Prepare at least 30 minutes prior to use)

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
    (Add 1 drop of DAB per ml of substrate)
    Lot#_____ Exp. Date_____ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

Updated 08/24/06